### **ORIGINAL ARTICLE**

## Comparative analysis of antibacterial activity of *povidone iodine* and homoeopathic mother tinctures as antiseptics

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### **ABSTRACT**

**Objectives:** Antiseptics are used extensively in hospitals and other health care centers (clinics) to control the growth of microbes on living tissues. They are essential parts of infection control practices, especially in wound treatment and aid in the prevention of nosocomial infections. This study was done to evaluate and compare the antibacterial effectiveness of *povidone iodine* and eight commonly used homoeopathic mother tinctures.

**Materials and Methods:** The present study was conducted in the Faculty of Pharmacy and alternative Medicine, The Islamia University of Bahawalpur. All the selected Homeopathic mother tinctures were purchased by Masood Homeopathic Pharmaceuticals, Pakistan. Prepared PYODINEâ (*povidone iodine*) solution 10% w/v, batch no. 084F4 used as positive control while 70% ethanol was used as negative control in each test. These tinctures were tested against three clinically important human skin-pathogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes,* and *Pseudomonas aeruginosa*) by disc diffusion method with *povidone iodine* as positive standard.

**Results:** Seven out of eight mother tinctures showed more or less antibacterial activity; some of them have high activity than *povidone iodine*. *Rhus glabra* had maximum activity (zone of inhibition 32 mm) against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* than *povidone iodine* and among all the tested mother tinctures.

**Conclusion:** This study confirms the antibacterial activity and more effectiveness of *Thuja occidentalis* and *Rhus glabra* mother tinctures than *povidone iodine*. The other tested mother tinctures also have antibacterial activity against tested bacteria, except *Echinacea*.



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### **INTRODUCTION**

Skin and soft tissue infections (SSTIs) are conditions produce considerable morbidity and affect life quality. Every year millions of people around the world experience SSTIs of both acute and chronic nature. A fascinating way of treating superficial skin This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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infections or wounds is through the use of topical antimicrobials or antiseptics.<sup>[1]</sup>

Antiseptics can be applied to kill or inhibit microorganisms' growth and have the ability to target multiple microbes. However, a common problem is to select the appropriate antiseptics because different pathogens respond in different way to different antiseptics.<sup>[2]</sup> Increasing bacterial resistance to antibiotics makes the management of superficial skin infections a major medical challenge. Antiseptics have broader spectrums of antimicrobial activity and a reduced potential for selection of bacterial resistance, relative to antibiotics. Therefore, antiseptics are proper alternatives to antibiotics for the prevention and treatment of superficial skin infections. From widely used antiseptics, povidone iodine has a particularly broad spectrum of antimicrobial activity that includes Gram-positive and Gram-negative bacteria, bacterial spores, fungi, protozoa, and viruses. Pyodine is used commonly in preoperative preparations, postoperative cleansing, open wounds, and cuts dressings.<sup>[3]</sup>

Mother tinctures play a role in homoeopathic prescribing particularly for their organotrophic effects.<sup>[4]</sup> Homoeopathic mother tinctures are commonly used by homoeopathic physicians at their clinics for various skin infections, chronic ulcerative conditions, and wound infections as topical antiseptics.<sup>[5]</sup> Homoeopathic tinctures are not antiseptic but bacteriostatic; they do not permit bacteria to flourish in their presence. They do not destroy epithelial tissues and the white blood cells which are the body's first line of defense against infection. Wound treated homoeopathically at the outset rarely become infected, and clinical observations demonstrate that healing is clean and occurs nearly 50% more rapidly than under traditional allopathic or orthodox approaches.<sup>[6]</sup>

The following homoeopathic plant mother tinctures were selected for the antibacterial testing.

Arnica montana from the family Asteraceae mostly used for bruises, sprains, and muscles pain.<sup>[7,8]</sup> It improves the local blood supply and speeds up the healing. It is anti-inflammatory and helps to increase the rate of reabsorption of internal bleeding.<sup>[9]</sup>

*Baptisia tinctoria*, a plant of *Leguminosae* family, is a strong antiseptic and immune stimulant. It is

considered effective in infected nipples and infected skin particularly.<sup>[10]</sup> A study showed moderate antibacterial activity of *B. tinctoria* and *Berberis vulgaris* against *Staphylococcus aureus*.<sup>[11]</sup>

*Berberis aquifolium* and *Berberis vulgaris* being part of *Berberidaceae* family have a considerable reputation as an antiseptic, blood purifier, and tissue cleanser and mostly used for skin diseases such as psoriasis, eczema, acne, and cold sores.<sup>[12]</sup>

*Calendula officinalis* is the most frequently used plant in various homoeopathic and herbal topical applications such as ointments, lotions, sprays, and dressings in cases of different skin problems such as injuries, lacerations, wounds, and ulcers in various studies and research works; *Calendula* is a great wound healer, antiseptic, and tissue proliferator.<sup>[13,14]</sup>

*Echinacea angustifolia* is a medical immune enhancer for skin diseases and general infections. It is valued by North American Indians of the USA. It became famed for cleaning and healing suppurative wounds.<sup>[15]</sup> Homoeopathically, it is used in cases of blood poisoning and septic conditions, also in cases of recurring boils, erysipelas, malignant ulcers, and gangrene.<sup>[16]</sup> However, a study showed that *Echinacea angustifolia* has no antibacterial effect against various human pathogenic bacteria.<sup>[17]</sup>

*Rhus glabra* member of *Anacardiaceae* family is known for its use in various skin eruptions and putrescent conditions with tendency to ulceration.<sup>[7]</sup>

*Thuja occidentalis* belongs to *Cupressaceae* family. It is used in ulcers, fig warts, boils, and chilblains;<sup>[18,19]</sup> also in cases of polyps, epithelioma, tubercles, carbuncles, and ulcers especially at the anorectal region; and also in skin eruptions only at the covered parts.<sup>[20]</sup> The present study is an attempt to evaluate and compare the antibacterial activity of above-mentioned homoeopathic plant mother tinctures with *povidone iodine*.

### **MATERIALS AND METHODS**

The present study was conducted in the Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur. All the selected homoeopathic mother tinctures were purchased by Masood Homoeopathic Pharmaceuticals, Pakistan. Prepared *PYODINE*<sup>®</sup> (*povidone iodine*) solution 10% w/v, batch no. 084F4 was used as positive control while

70% *ethanol* was used as negative control in each test.

### Preparation of Inoculum

Primary culture plate of the selected three bacteria was made by streaking the swab of the KWIK-STICK containing the respective bacterial strain on the fresh sterilized nutrient agar plate. A few colonies from the overnight primary culture plate of *Streptococcus pyogenes*, *Staphylococcus aureus, and Pseudomonas aeruginosa* were suspended in 10 ml sterile nutrient broth separately for three bacteria, and the solution was adjusted to the 0.5 McFarland equivalence turbidity standard.

### **Antibacterial Susceptibility Test**

All the selected homoeopathic mother tinctures along with *povidone iodine* were screened against three pathogenic bacterial strains. The tested organisms were *Staphylococcus aureus* ATCC-6538, *Streptococcus pyogenes* ATCC-19615, and *Pseudomonas aeruginosa* ATCC-9027 obtained from Microbiologics Inc., Minnesota, USA.

For testing the antibacterial activity, the disk diffusion method was used. Nutrient agar plates were made by pouring (15 ml) sterilized nutrient agar medium and allowed to solidify for a few minutes in aseptic condition. A volume of 60  $\mu$ l of prepared *inoculum* was poured into each plate. With the help of L-shaped glass rod spreader, the poured inoculum is spread on the whole surface of the agar uniformly. Filter paper discs of 6 mm diameter loaded with 10 µl of one selected mother tincture, povidone iodine and 70% ethanol separately were placed on the surface of the bacteria seeded nutrient agar medium in the respective labeled area. All the plates were incubated in an inverted position for 24 h at 37°C. After incubation, the clear zone surrounding each disc (zone of inhibition) was measured with the help of Vernier caliper. The work performed in triplicate for each mother tincture against each bacterial strain.

### **Statistical Analysis**

Analysis of variance was applied for statistical analysis of results. P < 0.05 was considered to be statistically significant.

### **RESULTS AND DISCUSSION**

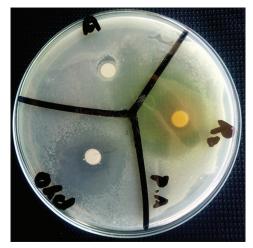
Table 1 shows the broad antibacterial activity of *povidone iodine* against all the three bacteria ranging 20–25 mm and had a maximum activity against *Staphylococcus aureus* while also

# Table 1: Results of mother tinctures, Povidoneiodine and alcohol against different bacterialstrains

Tested	Zone of inhibition (mm) Mean±SD		
material			
	Staphylococcus aureus	Streptococcus pyogenes	Pseudomonas aeruginosa
Povidone iodine	24±1.0	20±1.0	21±1.0
Alcohol	Nil	Nil	Nil
Arnica montana	15.3±0.76	15.3±0.76	9.3±0.76
Baptisia tinctoria	12.3±0.76	12.3±0.76	10.3±0.76
Berberis aquifolium	14±1.0	6.3±0.76	7.3±0.76
Berberis vulgaris	21.3±0.76	13±1.0	18.5
Calendula officinalis	19±1.0	20.6±0.66	11±1.0
Echinacea angustifolia	Nil	Nil	Nil
Rhus glabra	18.3±0.76	21.6±0.66	32.3±0.76
Thuja occidentalis	20.3±0.76	26.3±0.76	24.6±0.66

SD: Standard deviation

Streptococcus pyogenes effective against and Ps<mark>eu</mark>domonas aeruginosa. Rhus glabra had maximum zone of inhibition (32 mm) against Streptococcus pyog<mark>en</mark>es and Pseudomonas aeruginosa than pyodine and had also higher activity among all the tested mother tincture against Pseudomonas aeruginosa. [Figure 1] Thuja occidentalis was found to be most active than *pyodine* against *Streptococcus* pyogenes and Pseudomonas aeruginosa [Figure 2] and had also higher activity among all the tested mother tincture against Streptococcus pyogenes. Calendula officinalis was found to be more active than pyodine against Streptococcus pyogenes (20.6 mm) [Figure 3] while Berberis vulgaris had maximum activity among all the mother tinctures against Staphylococcus aureus (21.3 mm) [Figure 4]. Arnica showed moderate antibacterial activity (15.3 mm) against Streptococcus pyogenes and Staphylococcus aureus while showed 9 mm zone of inhibition against Pseudomonas aeruginosa. Baptisia showed 10.3 mm zone on inhibitions against Streptococcus pyogenes and Staphylococcus aureus while showed 9 mm zone of inhibition against Pseudomonas aeruginosa. Berberis aquifolium had also moderate activity against all tested bacteria with zone of inhibitions of 14.3 mm, 6.3 mm, and 7.3 mm against Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa, respectively. Echinacea



**Figure 1:**Antibacterial activity of *Rhus glabra* against *Pseudomonas aeruginosa*. A = alcohol, Pyo = pyodine, R = *Rhus glabra*, P.A = *Pseudomonas aeruginosa* 

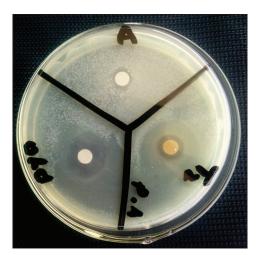
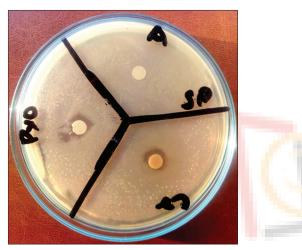


Figure 2: Antibacterial activity of Thuja occidentalis against Pseudomonas aeruginosa. A = alcohol, Pyo = pyodine, T= Thuja occidentalis, PA= Pseudomonas aeruginosa



**Figure 3:** Antibacterial activity of *Calendula officinalis* against Streptococcus pyogenes. A = alcohol, Pyo = pyodine, C = *Calendula*, S.P = Streptococcus pyogenes

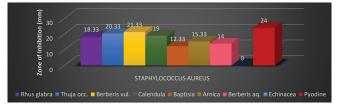


**Figure 5:** Comparison of homoeopathic mother tinctures with *povidone iodine* against *Pseudomonas aeruginosa*.Values are expressed as mean (n = 3). Diameter of disk was 6 mm

*angustifolia* had no activity against any bacteria and the result was in line with another study that also showed no antibacterial activity against pathogenic bacteria.<sup>[17]</sup> The diameters of growth of inhibitions of different mother tincture were 32.3 mm. *Povidone iodine* showed diameters of inhibitions in a range of 20–24 mm. There was statistically significant



**Figure 4:** Antibacterial activity of Berberis vulgaris against Staphylococcus aureus. A = alcohol, P = pyodine, Be = Berberis vulgaris, S.A = Staphylococcus aureus



**Figure 6:** Comparison of homoeopathic mother tinctures with *povidone iodine* against *Staphylococcus aureus*. Values are expressed as mean (n = 3). Diameter of disk was 6 mm

difference between the zone of inhibition values of *povidone iodine* and mother tinctures (P < 0.05) [Figures 5-7].

### CONCLUSION

Rhus glabra and Thuja occidentalis (against two bacterial strains) and Calendula officinalis (against



**Figure 7:** Comparison of homoeopathic mother tinctures with *povidone iodine* against *Streptococcus pyogenes*. Values are expressed as mean (n = 3). Diameter of disk was 6 mm

one bacterial strain) exhibited higher efficacy than *pyodine*. These three homoeopathic mother tinctures showed excellent zone of inhibition and were found to be more effective than *pyodine*, while *A. montana*, *B. tinctoria*, *Berberis vulgaris*, and *Berberis aquifolium* were found to be moderately effective against all the bacteria tested. Only *Echinacea angustifolia* showed no activity against any of the three bacteria. Hence, most of the homoeopathic mother tinctures proved to be effective in inhibiting the *in vitro* growth of three clinically important skin-pathogenic bacteria, *Staphylococcus aureus*, *S. pyogenes*, and *P. aeruginosa*.

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### **Conflicts of Interest**

There are no conflicts of interest.

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### संक्रमणरोधी के रूप में पोविडोन आयोडीन और होयोपैथिक मदर टिंक्चर की जीवाणुरोधी गतिविधि का तुलनात्मक विश्लेषण

### सार

**उद्देश्यः** संक्रमणरोधी दवाइयों का प्रयोग जीवित ऊतकों पर रोगाणुओं के विकास को नियंत्रित करने के लिए अस्पतालों और अन्य स्वास्थ्य देखभाल केन्द्रों (क्लीनिक) में बड़े पैमाने पर किया जाता है। वे घाव उपचार और नोसोकोमियल संक्रमण की रोकथाम में सहायता और विशेष रूप से संक्रमण नियंत्रण प्रथाओं के आवश्यक अंग हैं। यह अध्ययन पोविडोन आयोडीन और आठ आमतौर पर इस्तेमाल किये जानेवाले होम्योपैथिक मूलार्क की रोगाण रूरोधी क्षमता की तूलना और मूल्यांकन करने के लिए किया गया था।

**रचनाः** इन टिंक्चर की सकारात्मक मानक के रूप में पोविडोन के साथ डिस्क प्रसार विधि द्वारा तीन चिकित्सकीय दृष्टि से महत्वपूर्ण मानव त्वचा विकृति कारक बैक्टीरिया (स्टापय्लोकोकस आरियस, स्ट्रैप्टोकोकस पायोजेनेस और स्यूडोमोनास ऑरिगेनोसा) के विरुद्ध जांच की गई।

**परिणामः** आठ में से सात मदर टिंचर में कम या ज्यादा जीवाणुरोधी गतिविधियों से पता चला है कि उनमें से कई में पोविडोन आयोडीन की तुलना में उच्च गतिविधि है। पोविडोन आयोडीन और अन्य मदर टिंक्चर की तुलना में रुस ग्लेब्रा में स्ट्रेप्टोकोकस पायोजेन्स और स्यूडोमोनास ऑरियोगेनेसा के विरुद्ध अधिकतम गतिविधि थी।

निष्कर्षः इस अध्ययन से थूजा ऑक्सीडेंटलिस और रूसगलेब्र की रोगाणुरोधी गतिविधि और परम् प्रभावक्ता पोविडोन आयोडिन की अपेक्षा सुनिश्चित होती है। इचनाशिया को छोडकर परीक्षण में शामिल अन्य मदर टिंक्चरों में भी जीवाणूरोधी गतिविधियां पाई गयी।

### Análisis comparativo de la actividad antibacteriana de la yodo povidona y las tinturas madre homeopáticas como antisépticos

### RESUMEN

**Objetivos:** En los hospitales y en otros centros de salud (clinicas), se utilizan ampliamente los antisépticos para controlar el crecimiento de los microorganismos en tejidos vivos. Forman parte esencial de las medidas de control de las infecciones, en especial, en el tratamiento de heridas, así como en la prevención de infecciones nosocomiales. Este estudio se ha realizado para evaluar y comparar la eficacia antibacteriana de la yodopovidona y ocho tinturas madre homeopáticas habitualmente utilizadas.

**Diseño:** Estas tinturas fueron probados contra a tres bacterias dermatológicas humanas, clínicamente relevantes (*Staphylococcus aureus, Streptococcus pyogenes* y *Pseudomonas aeruginosa*) mediante el método disco en difusión. La yodopovidona se utilizó como estándar positivo.

**Resultados:** Siete de las ocho tinturas madre mostraron una actividad antibacteriana mayor o menor; la actividad de algunas de ellas fue superior a la de la yodopovidona. *Rhus glabra* presentó la actividad máxima (zona de inhibición: 32 mm) frente a *Streptococcus pyogenes* y *Pseudomonas aeruginosa* que fue superior a la de yodopovidona y la de todas las restantes tinturas madre examinadas.

**Conclusiones:** Este estudio confirma que la actividad antibacteriana y la eficacia de las tinturas madre de *Thuja occidentalis* y *Rhus glabra* son superiores a la de yodopovidona. Las restantes tinturas madre examinadas también mostraron una actividad antibacteriana, exceptuando *Echinacea*.